CHEMICAL RESEARCH, DEVELOPMENT & ENGINEERING CENTER

CRDEC-CR-080

FATE OF N,N-BIS-(2,4,6-TRICHLOROPHENYL)-UREA IN A FRESHWATER SEDIMENT

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May 1990

U.S. ARMY ARMAMENT MUNITIONS CHEMICAL COMMAND

Aberdeen Proving Ground, Maryland 21010-5423

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1990 May	Final, 88 May - 88 Oct

4. TITLE AND SUBTITLE
Fate of N,N-Bis-(2,4,6-Trichlorophenyl)-Urea in a Freshwater Sediment

5. FUNDING NUMBERS
PR-1C162622A553

6. AUTHOR(S)
Harvey, Scott D., Ph.D.; Dauble, Dennis D., Ph.D.; McVeety, Bruce D., Ph.D.; (continued on reverse)

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Pacific Northwest Laboratory, P.O. Box 999, Richland, WA 99352

8. PERFORMING ORGANIZATION REPORT NUMBER
CRDEC-CR-080

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
CDR, CRDEC, ATTN: SMCCR-RST-E, APG, MD 21010-5423

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
COR: Randall S. Wentsel, SMCCR-RST-E, (301) 671-2036

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

A primary purpose of this study was to determine whether CC-2 was transformed in sediment to the known environmental hazard 2,4,6-trichloroaniline (TCA). Another purpose of this work was to confirm the previously determined water solubility, and hence, the estimated K_{mw} value. Previous studies failed to address the possibility of microbial transformation of CC-2 to TCA, or were confounded by the formation of TCA as an artifact formed by gas-chromatographic analysis. The first task was to analyze sediments from Canal Creek for CC-2 and TCA. Soxhlet and ultrasonic extraction techniques were employed followed by analysis by HPLC. Secondly, the susceptibility of CC-2 towards microbial degradation was assessed by examining experimental sediments spiked with two concentrations of CC-2. Analyses for CC-2, TCA, and other possible transformation products were conducted over a 2-month period. The third objective was to determine the water solubility of CC-2 over a range of environmental relevant temperatures. The results of this study reinforce the perception that CC-2 is environmentally immobile and resistant of both chemicals and biological alteration.

14. SUBJECT TERMS
Sediment
CC-2
Environmental fate

15. NUMBER OF PAGES
31

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

6. Author(s) (continued)

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EXECUTIVE SUMMARY

Past studies have indicated the presence of N,N-bis-(trichlorophenyl)urea (CC-2) in concentrations as high as 388 ppm in sediments from Canal Creek. These studies also indicated that this compound has a low water solubility (0.082 ppm at 25°C) and a correspondingly large K_{ow} (>10^5). These properties, as well as the known stability of CC-2 towards chemical hydrolysis, point to the immobility and persistence of this compound in the aqueous environment. A primary purpose of this study was to determine whether CC-2 was transformed in sediment to the known environmental hazard 2,4,6-trichloroaniline (TCA). Another purpose of this work was to confirm the previously determined water solubility, and hence, the estimated K_{ow} value. Previous studies failed to address the possibility of microbial transformation of CC-2 to TCA, or were confounded by the formation of TCA as an artifact formed by gas-chromatographic analysis.

Three tasks were conducted for this study. The first task was to analyze sediments from Canal Creek for CC-2 and TCA. Soxhlet and ultrasonic extraction techniques were employed followed by analysis by HPLC. Secondly, the susceptibility of CC-2 towards microbial degradation was assessed by examining experimental sediments spiked with two concentrations of CC-2. Analyses for CC-2, TCA, and other possible transformation products were conducted over a 2-month period. The third objective was to determine the water solubility of CC-2 over a range of environmentally relevant temperatures.

Analysis of Canal Creek sediments indicated that CC-2 was present at a concentration of 77 ± 5 ppm. This value is within the range of concentrations observed in previous studies. TCA was not observed in sediments from Canal Creek. Experimental sediments containing CC-2 exhibited stable concentrations of this compound over a 2-month period. TCA was absent from the water above the experimental sediments at the conclusion of the study and was not observed in significant quantities within the sediments. These results provide evidence that CC-2 is resistant towards microbial transformation. Water solubilities determined by a dynamic coupled column liquid chromatographic technique were 0.019 ± 0.002, 0.052 ± 0.003, and 0.084 ± 0.004 ppm (w/v) at 5, 15, and 25°C, respectively. The value obtained at 25°C compares favorably with the value of 0.082 ppm obtained in an independent study. Collectively, the results of this study reinforce the perception that CC-2 is environmentally immobile and resistant to both chemical and biological alteration.

Several studies have shown that benthic organisms can bioaccumulate hydrophobic compounds from sediments. Further studies should be conducted to assess the potential for these organisms to serve as a source for propagation of CC-2 through food chain transfer.

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PREFACE

The work described in this report was authorized under Project No. 1C162622A553, CB Defense and General Investigation. This work was started in May 1988 and completed in October 1988.

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CONTENTS

LIST OF FIGURES AND TABLES

Figures

Tables

FATE OF N,N-BIS-(2,4,6-TRICHLOROPHENYL)-UREA IN A FRESHWATER SEDIMENT

1. INTRODUCTION

In 1981, the compound N, N-bis-(2,4,6-trichlorophenyl)-urea (CC-2) was found in surface sediments of Canal Creek, an estuarine system within the U.S. Army Aberdeen Proving Grounds, Maryland. Initial gas-chromatographic studies by Hydroponics Corporation showed that this compound undergoes thermal degradation within the injection port to form 2,4,6-trichloroaniline (TCA) and 2,4,6-trichlorophenyl isocyanate (Bruns 1983). Therefore, analyses conducted by gas chromatographic techniques were incapable of discriminating between TCA arising from thermal degradation of CC-2, or TCA originating from the possible biodegradation of CC-2. Analysis of sediments from Gunpowder River generally showed a low concentration of CC-2 (< 12 ppm); however, sediments collected from Canal Creek showed concentrations as high as 388 ppm (Bruns 1983). In a study by Dennis (1983), a liquid-chromatographic method was utilized to study sediment cores collected from Canal Creek. The concentration of CC-2 was found to rapidly decrease from a maximum of 100-300 ppm in the top 2 inches of sediment to below the detection limit of 5 ppm in sediments deeper than 6 inches. No mention regarding the presence or absence of TCA in sediments from Canal Creek was made in this report (Dennis 1983).

To assess the environmental mobility of CC-2, studies by Dennis (1983) focused on determining the water solubility of this compound in aqueous phosphate buffer. The results indicated a 95% confidence interval between 0.053 and 0.127 with a geometric mean of 0.082 ppm for the solubility of CC-2 at 25 °C. Additional studies indicated that the octanol/water partition coefficient was greater than 10^5 (Dennis 1983). These properties along with the known stability of CC-2 towards chemical hydrolysis point to the persistence of this compound in the aqueous environment. However, additional studies were needed to determine the fate of this compound in sediments. Therefore, we initiated investigations to verify and extend the available water solubility data (Dennis 1983), and to determine the potential of this compound to degrade in freshwater sediments.

2. MATERIALS AND METHODS

2.1 SYNTHESIS AND ANALYSIS OF CC-2

2.1.1 Chromatographic Conditions for CC-2 Determinations

Thermal lability of CC-2 precluded the use of gas-phase analytical techniques for the quantitation of this compound in sediment samples. Thus, the method chosen for the analysis of CC-2 was high performance liquid chromatography. The chromatographic system consisted of two Waters pumps (model M-6000A) controlled by a Waters model 660 solvent programmer. The standards and sediment extracts were injected by a Wisp 710 automatic injector onto a Beckman Ultrasphere 5-μm octadecyl silica column and the components separated by a linear solvent program at a flow rate of 1.0 mL/min. The solvent system was water/acetonitrile, with a 15 min gradient from 60% to 100% acetonitrile (ACN). Components were detected by UV absorption at either 230 or 254 nm (Schoeffel GM 770 detector). Peak areas obtained from a Hewlett Packard 3390A integrator were used for quantitative measurements. In one instance, a photodiode array detector (Waters model 990) was utilized to obtain UV/VIS spectra of the components extracted from Canal Creek sediment obtained from the Aberdeen Proving Ground.

2.1.2 Synthesis of CC-2

The synthetic strategy chosen was that described by Dennis (1983). Briefly, 0.15 moles of urea, 0.047 moles of 2,4,6-trichloroaniline, and 80 mL of glacial acid was brought to reflux in a 250-mL round-bottomed flask. Concentrated sulfuric acid (15 g) was slowly added over a 2-hr period. Reflux was allowed to continue for an additional 3 hrs at which time the crude product was isolated by suction filtration. After washing with hot water, the product was air dried and analyzed by HPLC. The crude product was repetitively mixed with methanol in an ultrasonic bath, centrifuged, and decanted in order to remove unreacted TCA starting material. Purity of the crude and purified product was assessed by liquid chromatography. Verification of structure was possible by examining the 70-ev electron impact direct inlet probe mass spectrum.

Stock solutions for the generation of a standard curve of CC-2 were prepared by diluting a concentrated stock solution containing 8.63 mg CC-2/10 mL N,N-dimethylformamide (DMF) with ACN. Integration of peak areas resulting from the analysis of freshly prepared standards allowed for construction of a standard curve. The validity of the standard curve was periodically checked throughout the study by comparing

peak areas resulting from injections of standards containing known concentrations of CC-2 to the original standard curve.

2.1.3 Determination of CC-2 in Canal Creek Sediments

Sediment samples were obtained from Canal Creek in September 1988 and shipped to the Pacific Northwest Laboratory. A subsample was oven-dried at 60 °C and sent to the Oregon State University (OSU) Soil Testing Laboratory in Corvallis, Oregon for analysis of particle size and carbon content. Organic carbon content was 2.4 ppm and particle size distribution (by weight) was: > 50 µm, 56 %, 50 to 2 µm, 28 %, and < 2 µm,16 %.

Two different methods were compared in their ability to extract CC-2 from Canal Creek sediments. In the first method, 5.0 g of sediment was Soxhlet extracted for 20 hrs with 200 mL of acetonitrile (ACN). After extraction, 2.5 mL of dimethylformamide (DMF) was added and the solution filtered through a 0.2 µm Nylon 66 filter. The volume was then reduced to 50.0 mL by rotary evaporation and the resulting solution analyzed by reverse phase HPLC for the presence of CC-2 and trichloroacetic acid (TCA). The second method utilized ultrasonication of 0.5 g of sediment in 5.0 mL of 5 % DMF in ACN as described by Dennis (1983). After 15 min of sonic agitation, the sediment was removed by centrifugation for 5 min at 483 x *g*. The supernatent was decanted and subsequently centrifuged for 5 min at 4354 x *g* prior to analysis by HPLC.

2.2 CC-2 BIODEGRADATION STUDY

2.2.1 Sediment Collection and Preparation

Control sediments for biodegradation studies were obtained near McNary Dam on the Columbia River. Surface sediments were collected by scoop dredge, transported to the laboratory in Richland, Washington and stored at 4 °C. To prepare a uniform media for testing, sediments were dried at 60 °C for 72 hrs, then passed through a 0.25 mm mesh sieve. A subsample was sent to the Soil Testing Laboratory at OSU for analysis. Organic carbon content was 1.7 ppm and particle size distribution (by weight) was: > 50 µm (sand), 1 %, 50 to 2 µm (silt), 78 %, and < 2 µm (clay), 21 %.

Prior to initiating the aging study of CC-2 in McNary Dam sediments, a series of experiments were conducted in order to determine the recovery of CC-2 when added at various concentrations to McNary Dam sediment. Triplicate oven-dried sediment samples were spiked with a solution of CC-2 dissolved in DMF to final concentrations of 86.3, 51.8,

and 17.3 ppm CC-2 on a dry weight basis. The DMF solvent was removed under a 7 mtorr vacuum for a period of 2 hrs, at which time the sediment samples were subjected to ultrasonic extraction and HPLC analysis.

2.2.2 Experimental Design

Experimental sediments systems were spiked with either 80 or 20 ppm CC-2. The 80 ppm system was designed to mimic concentrations found in Canal Creek sediments, whereas the 20 ppm system was selected to minimize the possible toxic effects of the simulant on bacterial flora. The experimental design included duplicate 600-ml glass beakers representing blank, 20 ppm CC-2, and 80 ppm CC-2 sediments. The blank treatment contained 60 g dried sediment, 10 g fresh sediment (containing 70% water by weight), and 300 mL of Columbia River water. The 20 and 80 ppm treatments were prepared by spiking 35 g oven dried sediment with a CC-2 standard dissolved in DMF. The DMF was removed under vacuum for at least 2 hrs. Test sediments were then mixed with an additional 25 g of dried sediment and 10 g fresh sediment before 300 mL of Columbia River water was added. This resulted in a final sediment/water ratio of approximately 1:5 (Muir et al. 1983). The purpose of adding Columbia River water and fresh sediment was to seed the sediments with bacteria. The sediment in each beaker was mixed by stirring at low speed with a magnetic stir bar for 24 hrs and sediments were allowed to settle for 24 hrs before initial samples (time = 0) were collected. Two sediment cores were withdrawn from each beaker for chemical analysis. These sediment cores were about 0.5 i.d. by 1.5 cm and averaged 2.5 g dry weight. The interstitial water was removed by drying the sediments at 68 °C for 15 hrs. These samples were then subjected to ultrasonic extraction and HPLC analysis for TCA and CC-2. Additional core samples were obtained from each beaker at time = 13, 27, and 61 days for chemical analysis.

The test beakers were covered with a glass plate and placed on a lab benchtop at 21 ± 1 °C during testing. They were exposed to artificial lighting for about 12 hrs per day. All exposures were static, with no aeration. Columbia River water was periodically added during the test period to replenish the volume lost to evaporation. Dissolved oxygen measurements were made at 31 and 61 days using a oxygen electrode.

2.2.3 Analysis of Water for TCA

The methodology for analysis of TCA in the water above the experimental sediments consisted of preconcentrating hydrophobic compounds on a C-18 Sep-Pak precolumn. Initial studies tested for breakthrough and extraction efficiency by passing 100 mL of 0.10

ppm TCA in water through two Sep-Pak cartridges connected in series. The components on each cartridge were then independently eluted with 5.0 mL methanol (MeOH). The MeOH was subsequently reduced in volume under a stream of dry nitrogen to 1.0 mL. A 20 µl aliquot of this solution was analyzed by HPLC. The chromatographic system was the same as previously described with the exception that absorption at 254 nm was utilized for detection. Extraction efficiency was evaluated relative to the peak area resulting from a 20 µl injection of a 10 ppm methanol solution of TCA.

Analysis of the water above the t = 61 day sediments consisted of filtering the water from each beaker through a 0.2 µm Nylon 66 filter. A 100 mL aliquot of each water sample was then passed through two serially coupled Sep-Pak cartridges. Both Sep-Paks were subsequently eluted with methanol and analyzed for TCA.

2.3 DETERMINATION OF THE AQUEOUS SOLUBILITY OF CC-2

The liquid-chromatographic system utilized for the determination of CC-2 solubility consisted of two independent pumping systems (Waters model 6000A pumps) interconnected through a six-port valve (Rheodyne model 7000). One pump was used exclusively to deliver buffer (pH = 7.01 ± 0.04, 1 mM potassium phosphate) at a flow rate of 200 µl/min through the generator column (60 x 0.76 cm i.d.). A six-port valve (Rheodyne model 7000) situated after the generator column allowed for flow of saturated CC-2 buffer through either a Brown-Lee C-18 guard column (4.0 x 0.5 cm i.d.) or to waste. During the period that CC-2 was being stripped from the saturated buffer by the guard column, the generator column eluant was collected and the volume determined gravimetrically. Two pumps comprised the second pumping system and delivered isocratic mobile phase (70% MeOH, 30% H_2O) at a flow of 1.5 mL/min through an injector (Rheodyne model 7125) onto a 5 µm C-18 Nova-Pak radial compression analytical column. Flow of the mobile phase could be diverted *via* the six-port valve to by-pass the guard column or to back-flush the pre-concentrated components from the guard column onto the analytical column for separation and quantitation. Calibration standards and pre-concentrated solutes from the generator column were detected by UV absorption at 230 nm (Waters Model 450 detector). Integrated peak areas were provided by a Hewlett Packard 3390A integrator.

The generating column was a stainless steel tube (60 x 0.75 cm i.d.) packed with 60/80 mesh glass beads (Applied Science) containing a 1 % loading of CC-2. The beads were coated by stripping the solvent from a mixture of 0.5637 g CC-2 dissolved in 725 mL DMF containing 56.37 g of glass beads. To assure complete evaporation of DMF, the coated

beads were kept under vacuum (7 mtorr) overnight before the generating column was packed. The generating column was encased in cylinder through which water from a thermostated water bath was circulated to control the column temperature to within ± 0.5 °C.

3. RESULTS AND DISCUSSION

3.1 DETERMINATION OF PURITY AND SYNTHESIS OF CC-2

The purity of a sample of CC-2 (provided by the Chemical Research and Engineering Center, Aberdeen Proving Ground) was evaluated as a possible analytical standard for this work. Figure 3.1A shows a chromatogram of a 21 ppm solution of this material (250 µl of 8.35 mg CC-2/10 mL DMF diluted to 10 mL with ACN). It is immediately evident from this chromatogram that this material was not pure and not suitable for use in recovery studies. Therefore, it became necessary to synthesize CC-2 prior to initiation of the present studies. The chromatogram shown in Figure 3.1B shows that the crude product, after washing with hot water and air drying, contained a major constituent (retention time of 10.47 min) as well as an appreciable amount of 2,4,6-trichloroaniline (retention time of 11.63 min) starting material. Further purification was based on the preferential solubility of trichloroaniline in methanol. The product was sonicated in 15 mL of methanol, centrifuged and the supernatent discarded. This washing procedure was repeated twelve times before analytical assessment of purity was conducted by HPLC. The final 2.35 g of product represented a reaction yield of 24 %. The chromatogram shown in Figure 3.1C shows the analysis of a 10 ppm solution of final CC-2 product. As can be seen, this product was free of any detectable 2,4,6-trichloroanailine starting material or other UV-absorbing impurities. Additionally, a sample of the synthetic product was submitted for analysis by direct-probe mass spectrometry. The mass spectrum of the synthetic material is presented in Figure 3.2. The 70-ev electron impact mass spectrum shows a molecular ion at m/e of 416 and the expected superimposed mass spectra of trichloroaniline and trichlorophenyl isocyanate, thereby verifying the synthesis of the target compound. This product was used in all subsequent experiments contained in this report. It is noteworthy that the material provided by Aberdeen Proving Ground contained a compound with the same retention characteristics as CC-2. However, CC-2 is not the major constituent of this mixture.

3.2 DETERMINATION OF CC-2 IN CANAL CREEK SEDIMENTS

Prior to the quantitation of CC-2 in Canal Creek sediments, a standard curve for this compound was prepared (Figure 3.3). Reference to this standard curve forms the basis of quantitation of all portions of this report except for the determinations concerning the solubility of CC-2.

The chromatograms presented in Figure 3.4 compare the chromatographic profiles of Canal Creek sediment generated by the Soxhlet extraction method (Figure 3.4A) and

sonic extraction technique (Figure 3.4B). It is evident that both techniques generated similar profiles; however, the Soxhlet extraction method gave a lower analysis of CC-2 (62 ppm) when compared to the sonic extraction method. A triplicate analysis of Canal Creek sediment utilizing the sonic extraction technique gave an average CC-2 concentration of 77 ± 5 ppm.

Of primary importance regarding the analysis of these sediments was the determination of the presence or absence of TCA. The chromatographic profiles revealed a peak eluting with a retention time which was close to that of TCA. To determine whether this peak is due to TCA, a standard of TCA was co-injected with the Soxhlet extract from Canal Creek sediment. For comparison, the chromatogram of the Soxhlet extract is shown in Figure 3.5A with the co-injection of this extract and TCA presented in Figure 3.5B. In this comparison, the peak eluting immediately after CC-2 in the extract from Canal Creek and TCA do not co-elute. Thus, Canal Creek sediments do not show any detectable TCA. Interestingly, TCA was found in Canal Creek sediment samples which were oven dried at 140 °C for 10 hrs prior to analysis (Figure 3.5C). Evidently, this temperature was sufficient to initiate thermal degradation of CC-2 to TCA and trichlorophenyl isocyanate.

3.3 STABILITY OF CC-2 IN SEDIMENTS

In comparison to Canal Creek sediments, the particle size distribution of McNary dam sediments are different; however, the difference is mainly attributable to the sand *versus* silt fractions. The sediments from both areas contained similar amounts of the clay fraction and the organic carbon content is low. Thus, sorption properties of CC-2 should be similar for the two sediment types.

The recovery of different concentrations of CC-2 from McNary Dam sediments are shown graphically in Figure 3.6. The recovery from both the 86.3 and 51.8 ppm sediments was approximately 92 %, whereas recovery from the 17.3 ppm sediment averaged 81%. This initial experiment showed that analysis can proceed with good recovery from McNary Dam sediment spiked with a comparable concentration of CC-2 as Canal Creek sediments (77.4 ± 5 ppm). The recovery observed for the 17.3 ppm sediment was significantly lower than recoveries from the 86.3 and 51.8 ppm sediments (ANOVA, $p < 0.05$) and may reflect irreversible adsorption of CC-2 on the sediment.

Differences were observed between oxygen concentrations of blank and test beakers during the test (Table 3.1). In general, the beakers without CC-2 were near saturation at 31 and 61 days. In contrast, the test beakers had lower dissolved oxygen

concentrations at both measurement intervals (Table 3.1). It appears that the addition of CC-2 resulted in chemical oxygen demand that reduced oxygen concentrations in both the 20 and 80 ppm beakers through most of the test period.

TABLE 3.1 Comparison of Dissolved Oxygen (O_2) Concentrations in Duplicate Treatment Beakers During the Biodegradation Experiment

Treatment Beaker	Time (days)	[O_2] (mg/L)	% Saturation†
Control (a)	31	6.9	83.4
Control (b)	31	6.7	80.7
20 ppm (a)	31	2.6	31.6
20 ppm (b)	31	1.0	12.2
80 ppm (a)	31	1.8	21.8
80 ppm (b)	31	1.5	17 6
Control (a)	61	7.9	95.4
Control (b)	61	7.5	89.6
20 ppm (a)	61	5.1	61.8
20 ppm (b)	61	4.2	50.4
80 ppm (a)	61	4.2	50.3
80 ppm (b)	61	4.0	48.1

† Saturation values based on measurements taken at 25 °C

Representative chromatograms of sediments from a blank beaker (top) and an 80 ppm beaker (bottom) at the initiation of this study are shown in Figure 3.7. McNary Dam sediment shows no interfering compounds in the elution region of CC-2. Figure 8 shows representative chromatograms of an 80 ppm sediment at 14 (Figure 3.8A), 27 (Figure 3.8B), and 61 (Figure 3.8C) days. In general, the concentration of CC-2 remained stable throughout the time course of the experiment in both the 20 and 80 ppm sediments. Interestingly, a small peak which matches the retention time of TCA is observed in the sediments at 14, 27, and 61 days. This component is near the detection limit and does not increase in concentration as the sediments are aged with CC-2. Although this peak is not necessarily due to TCA, two possible explanations could account for the appearance of TCA in these sediment samples.

Either a very small amount of CC-2 is biodegraded to TCA, or a small fraction of CC-2 is thermally degraded by drying the sediments at 68 °C. In light of the constant peak areas of both CC-2 and this small peak throughout the study, as well as the absence of TCA in the water above the sediments (see below) at the conclusion of the study, it is most likely that this peak is due to thermal degradation of CC-2. A summary of the quantitative determinations of CC-2 is tabulated in Table 3.2. A peak with a retention time of 24.7 min was observed to appear in spiked McNary Dam sediments. A peak with identical retention time was also observed in sediment from Canal Creek. Although the chemical identity of this component remains obscure, the UV spectrum, as obtained on a photodiode array detector, shows absorbance maxima at 225 and 265 nm with a shoulder at 280 nm.

TABLE 3.2 Average CC-2 Concentrations ± Standard Deviations in Test Beakers During the 61-day Exposure Period. All Measurements in the Control Sediments (blank) Were Below the Detection Limit of 2.0 ppm.

	Exposure Duration			
Treatment	0 day*	13 day*	27 day†	61 day†
# of analyses	(n = 2)	(n = 2)	(n = 3)	(n = 3)
20 ppm (a)	15.3 ± 0.4	15.7 ± 0.6	15.1 ± 0.7	16.5 ± 0.9
20 ppm (b)	14.1 ± 0.2	17.1 ± 1.1	16.0 ± 1.0	18.2 ± 0.6
80 ppm (a)	68.1 ± 0.3	74.6 ± 0.3	70.9 ± 0.4	72.1 ± 1.1
80 ppm (b)	68.0 ± 0.3	78.4 ± 0.4	76.1 ± 6.5	71.2 ± 2.4

* average value ± range/2
† average value ± standard deviation

Figure 3.9 shows chromatograms comparing a 20 µl injection of 10 ppm solution of TCA (Figure 3.9A) with a 20 µl injection of 100 mL of 0.10 ppm TCA concentrated to 1.0 mL methanol by use of a Sep-Pak cartridge (Figure 3.9B). The extraction efficiency of the cartridge (as based on a comparison of peak areas) was 80%. An estimated detection limit of 10 ppb TCA should be realized by use of the above preconcentration scheme. Analysis of the second tandem Sep-Pak cartridge (Figure 3.9C) showed no detectable TCA indicating that breakthrough did not occur. Analysis of water above the sediments at the conclusion of the experiment showed no detectable CC-2, TCA, or other degradation products.

3.4 AQUEOUS SOLUBILITY OF CC-2

Previous work by Dennis (1983) utilized the ^{14}C urea derivative to determine the water solubility of CC-2 in mM phosphate buffer. Radiolabeled CC-2 was equilibrated with pH 7.0 phosphate buffer, and the radioactivity in the aqueous solution was determined in order to calculate the concentration of CC-2. The solubility determined by the radiocounting procedure was 0.082 µg/mL; however, the variability of the data was quite large (Dennis 1983). Although this method can be very sensitive, solubilities determined in this manner are plagued by a number of problems. One of the problems associated with this method is that any CC-2 existing in colloidal, micellar or particulate forms would cause an overestimate of the true water solubility of the analyte. Another problem associated with similar methods is the absorptive loss of compound on container surfaces (May and Wasik 1978; May et al. 1978).

An analytical methodology has been described by May and co-workers which circumvents the above problems (May and Wasik.1978; May et al. 1978). In this method, water or buffer is passed through a generating column containing glass beads coated with the analyte of interest. The low flow rates normally used as well as the high surface area of the beads assure that the emerging solution is saturated with analyte. The analyte can be preconcentrated from the saturated solution on a C-18 guard column and analyzed by HPLC. Additional advantages of this technique include chromatographic separation of interfering compounds and the short temporal lag in the generation of the saturated solution and the analysis of analyte (May and Wasik.1978; May et al. 1978). The latter advantage may be important if the analyte is not stable in aqueous solutions. As such, this technique represents a significant improvement in the existing methodology.

Figure 3.10 presents a standard curve generated on the chromatographic system used to determine solubilities. Temperatures chosen for this study included 5, 15 and 25 °C to bracket a large range of environmentally relevant temperatures. The water solubility data indicates a good relationship between temperature and solubility (Figure 3.11). A total of six determinations were made at each temperature. The solubility of CC-2 at 25 °C was determined to be 0.084 ± 0.004 ppm. This is in good agreement with the value of 0.082 ppm obtained by Dennis (1983). Although the average solubilities from the present study and the work of Dennis compare favorably, the precision attainable by techniques used in this study are much higher. The solubilities determined at 5 and 15 °C were 0.019 ± 0.002 and 0.052 ± 0.003 ppm, respectively. The least squares fit to this data has no theoretical significance other than allowing for extrapolation of solubility to other temperatures.

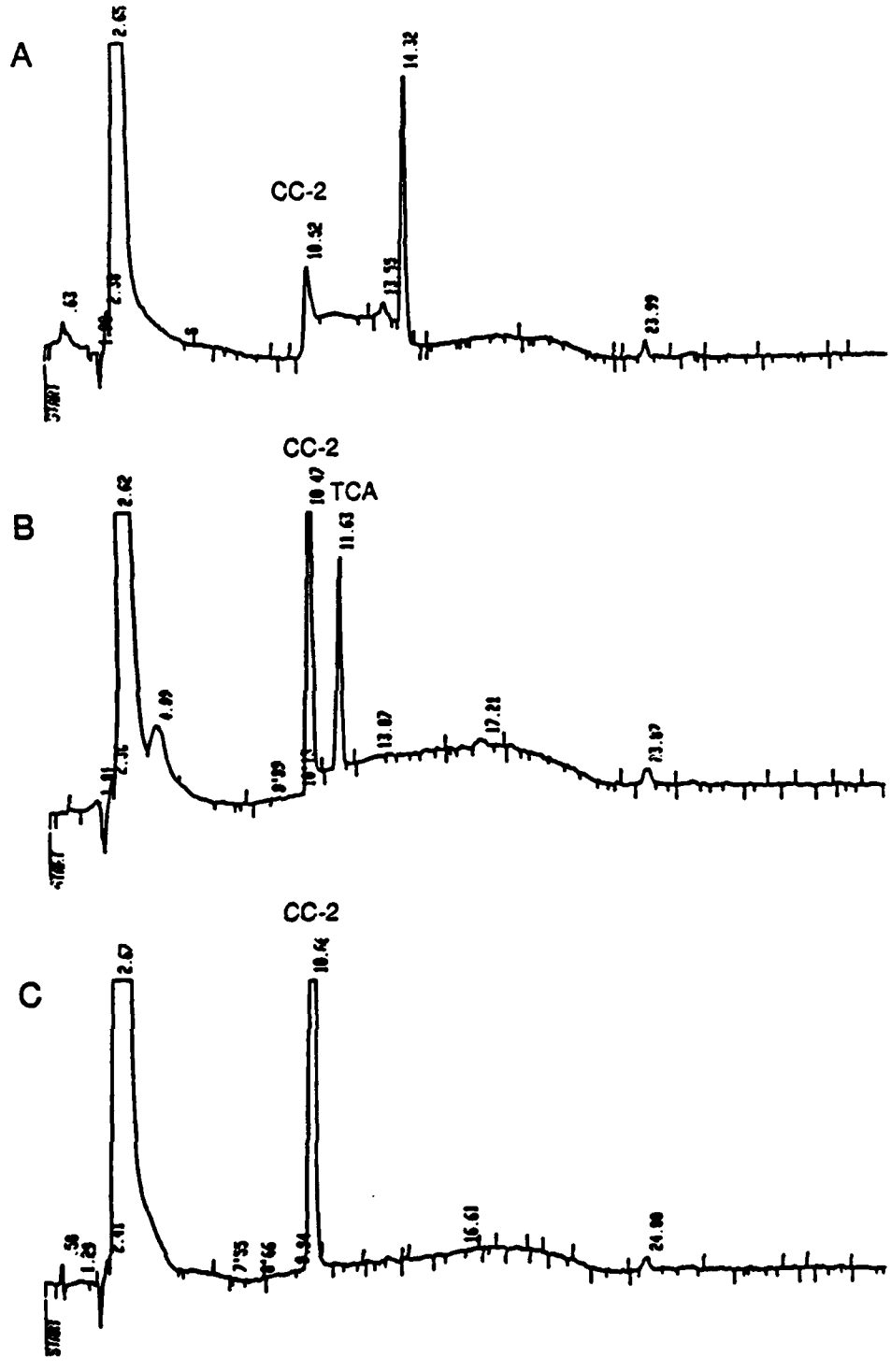

FIGURE 3.1 Chromatograms of: A) CC-2 Provided by Aberdeen Proving Ground, B) Crude Synthetic CC-2, and C) Purified CC-2 Product

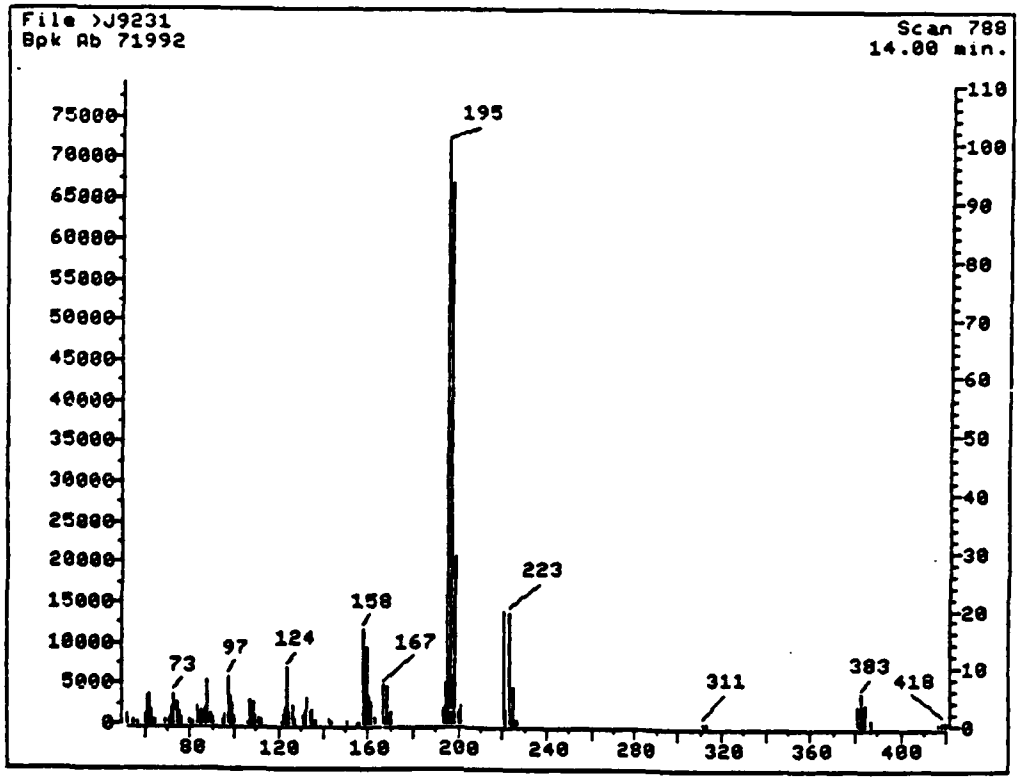

FIGURE 3.2 Mass Spectrum of Purified Synthetic CC-2 Product

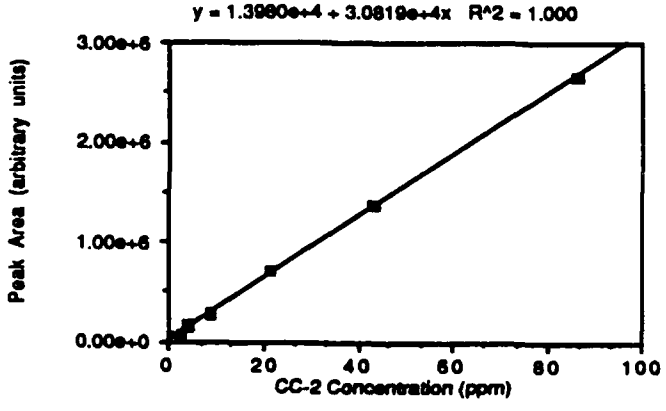

FIGURE 3.3 Standard Curve for CC-2

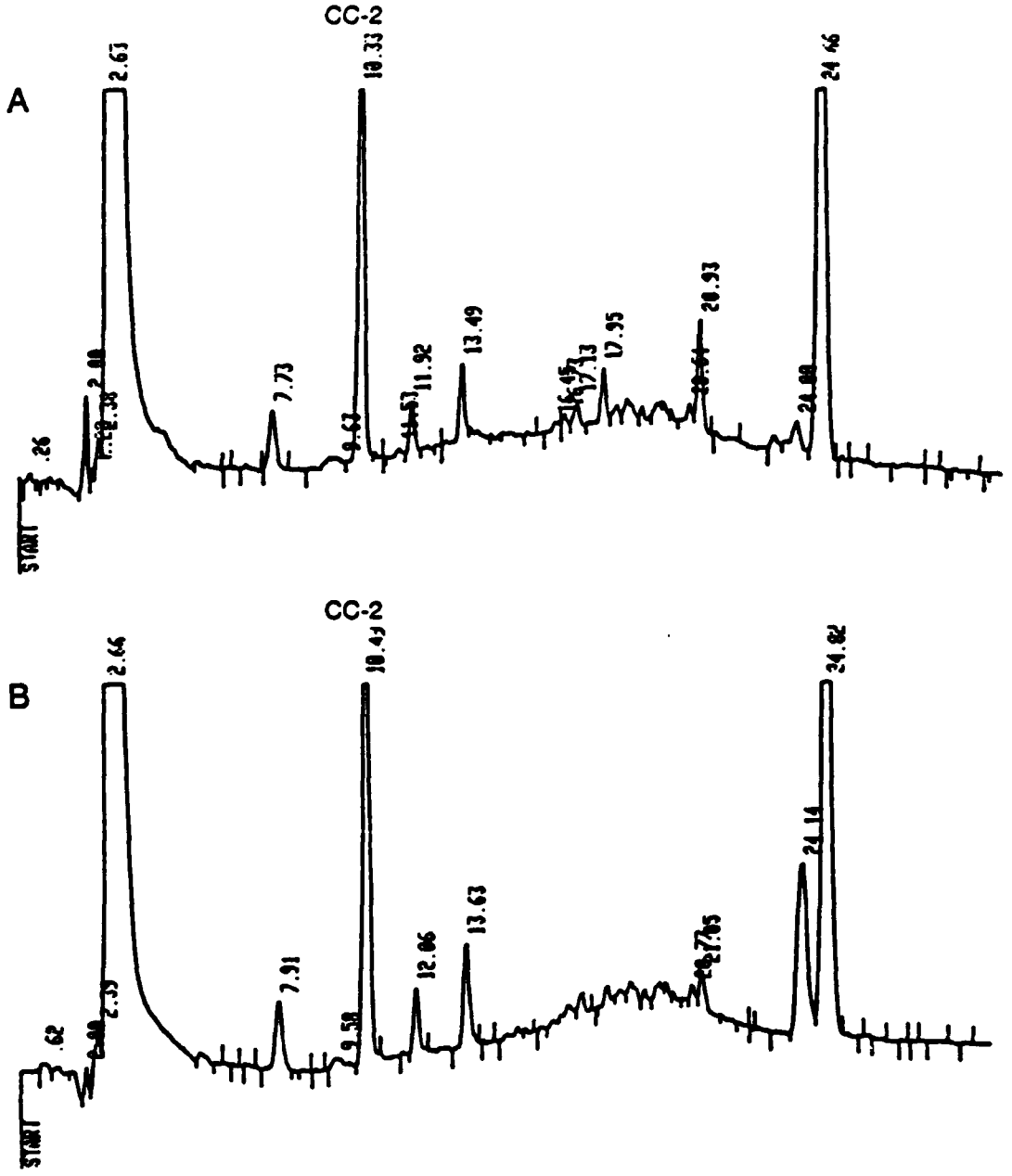

FIGURE 3.4 Chromatograms of Extracts from Canal Creek Sediment Obtained by the A) Soxhlet Extraction Method and B) Ultrasonic Extraction Method

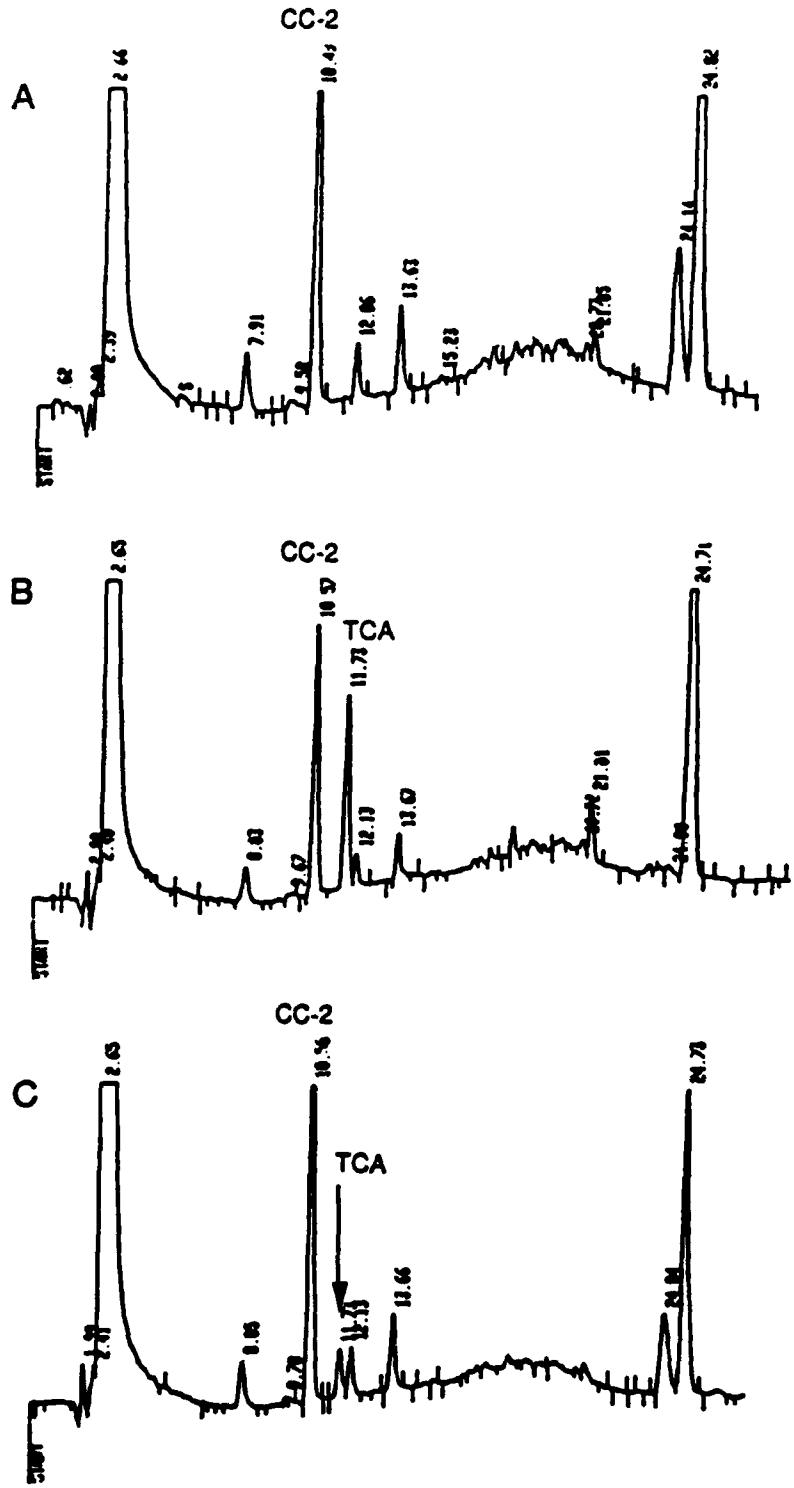

FIGURE 3.5 Chromatographic Profiles of Canal Creek Sediment Obtained from A) Soxhlet Extraction, B) a Co-injection of TCA and the Soxhlet Extract, and C) Sonic Extraction of Sediment which was Previously Dried at 140 °C for 10 hr

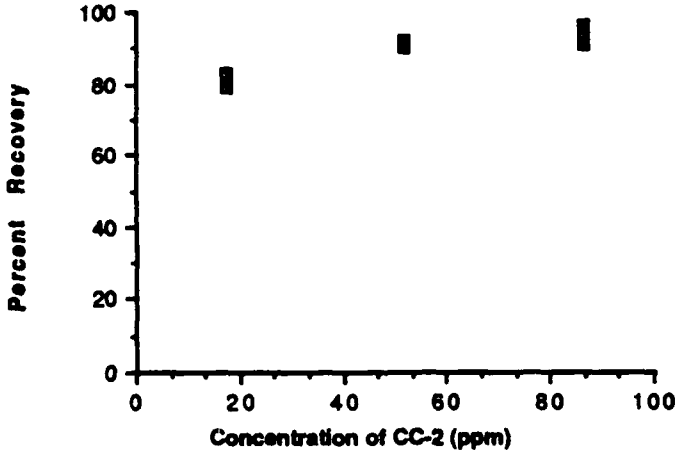

FIGURE 3.6 Recovery of CC-2 from McNary Dam Sediment

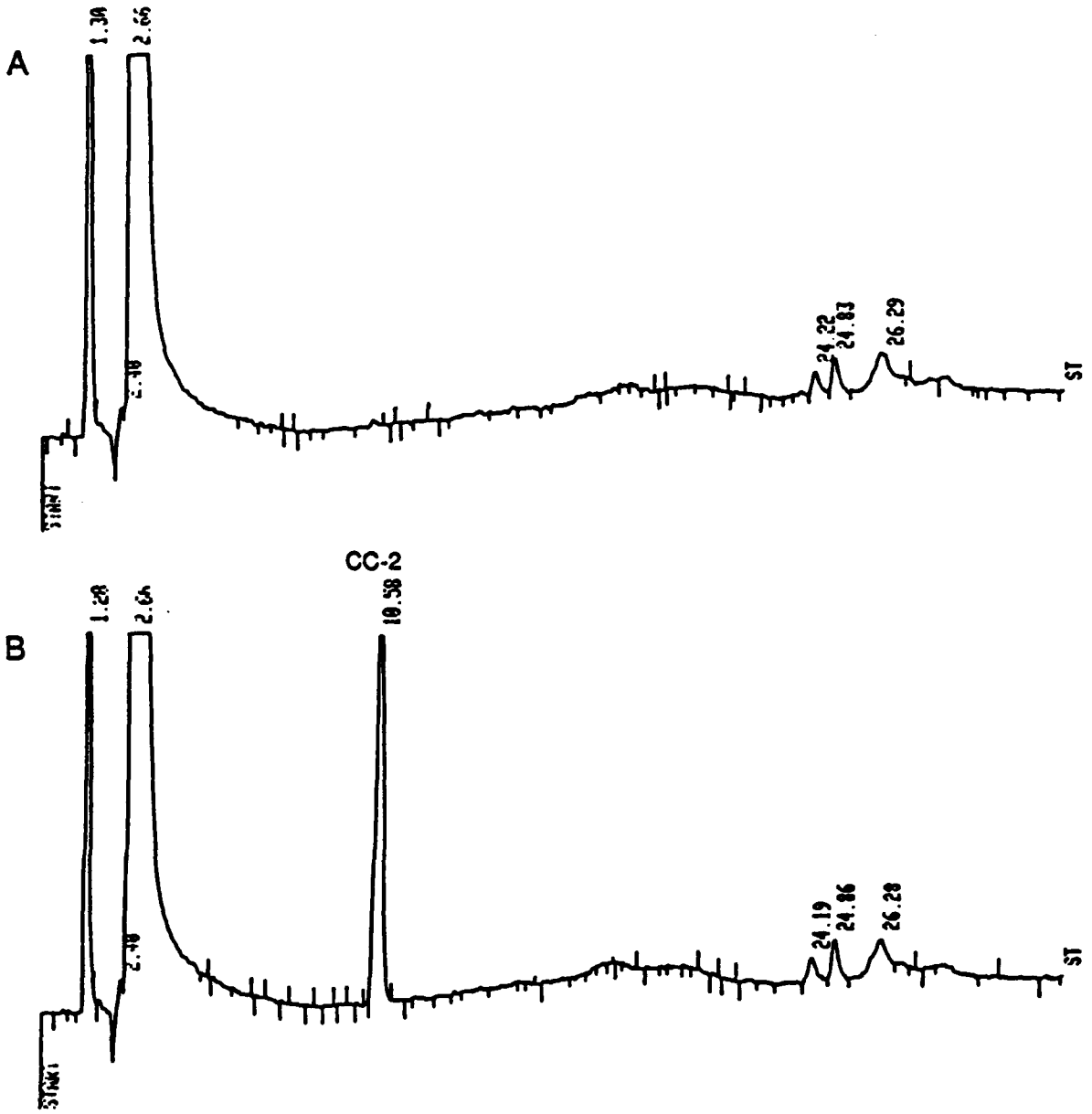

FIGURE 3.7 Chromatograms Illustrating: A) McNary Dam Sediment (blank), and B) McNary Dam Sediment Spiked with 80 ppm CC-2 (t = 0 days)

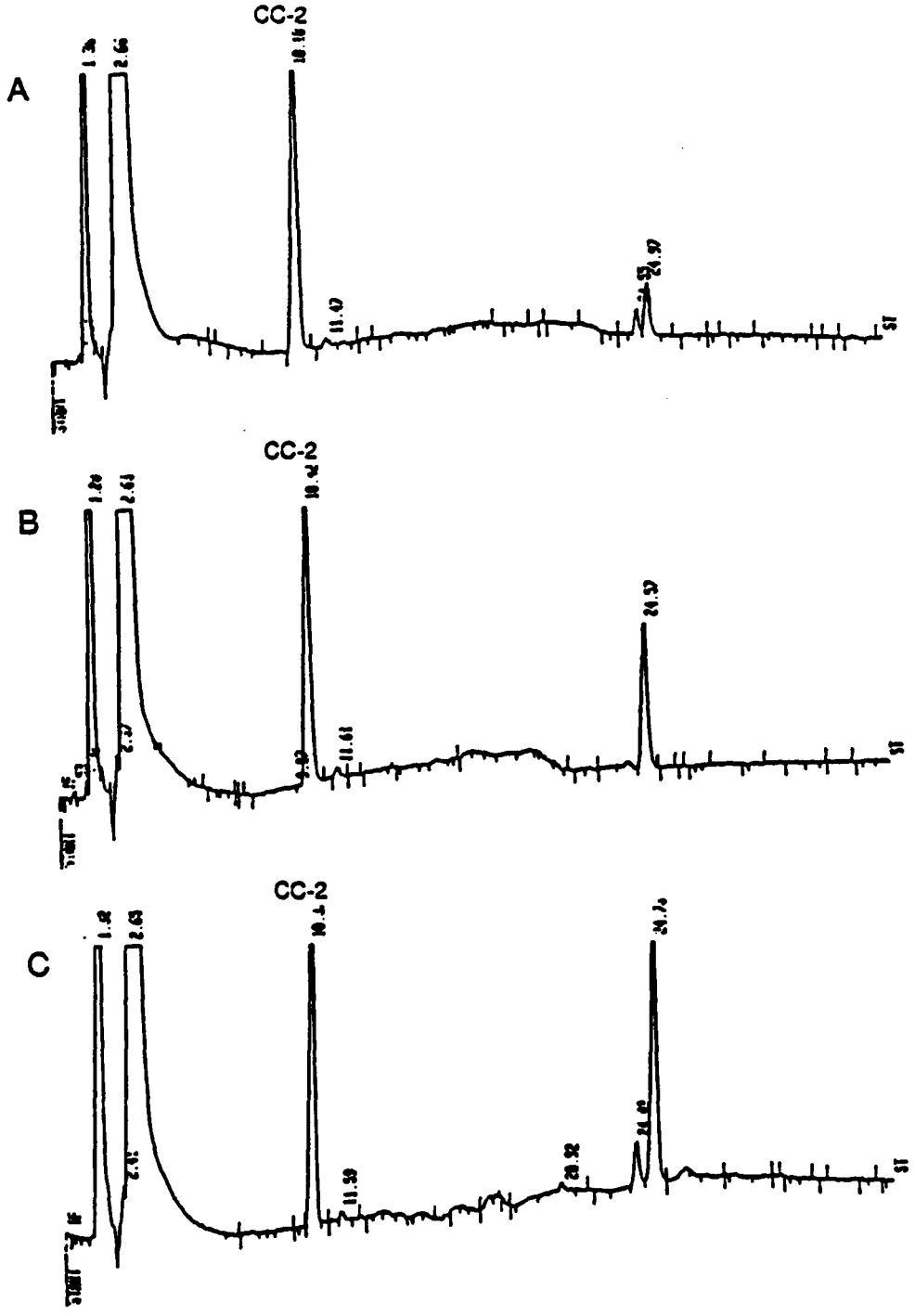

FIGURE 3.8 Chromatographic Profiles of McNary Dam Sediment Containing 80 ppm CC-2 at: A) 13, B) 27, and C) 61 Days

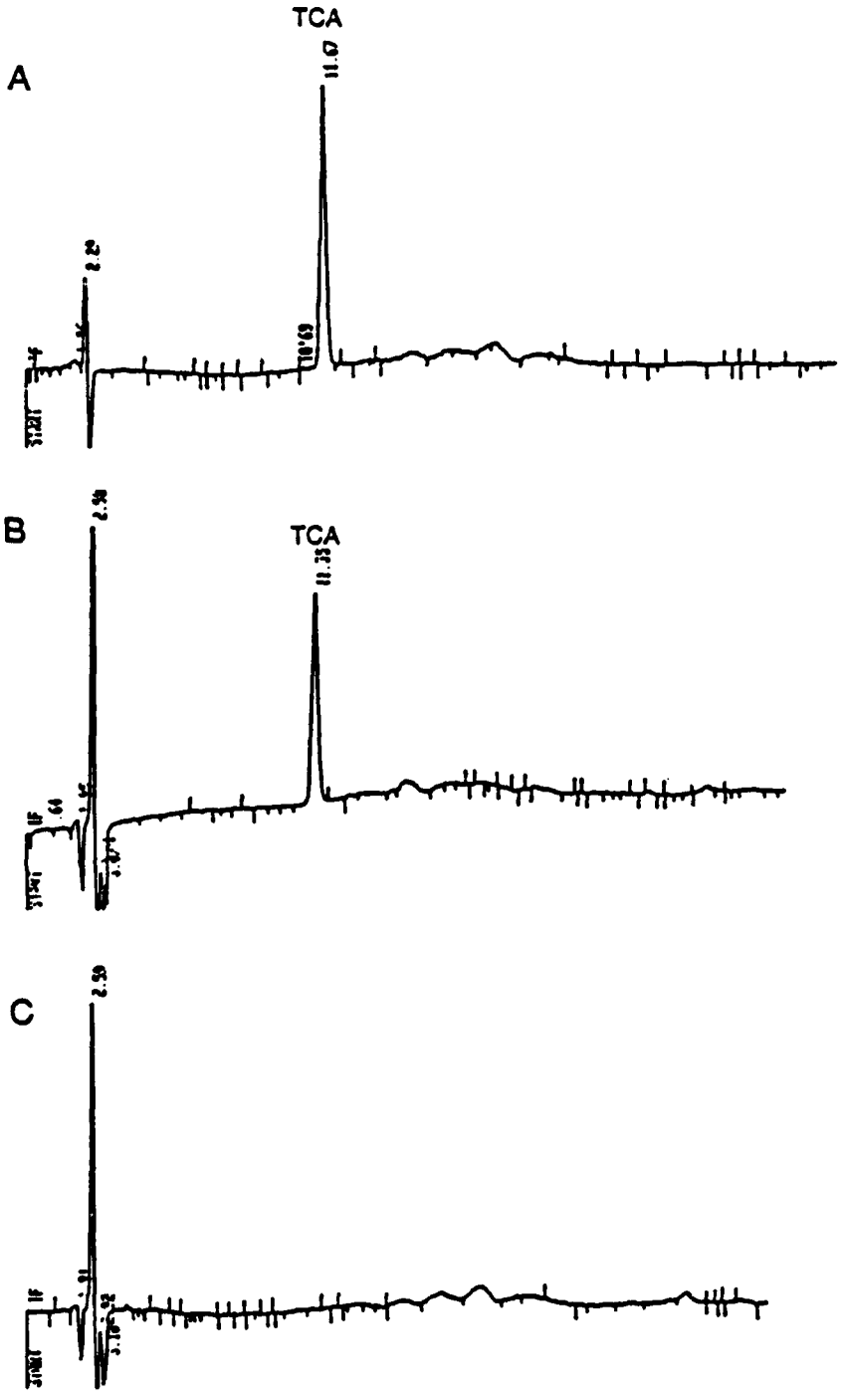

FIGURE 3.9 Chromatograms Displaying: A) Direct Injection of 10 ppm TCA, B) Extract Resulting from Preconcentration of 100 mL of 0.10 ppm TCA on the First C-18 Sep-Pak Cartridge, and C) Components Contained on the Second Tandem Sep-Pak Cartridge

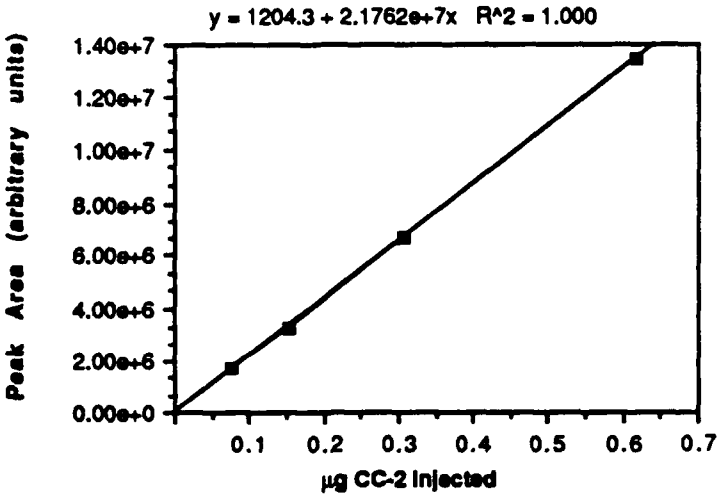

FIGURE 3.10 Standard Curve for CC-2 Solubility Studies

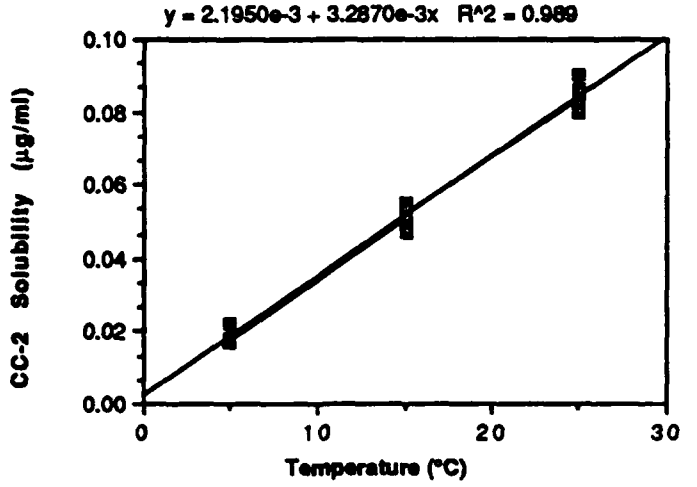

FIGURE 3.11 Solubility of CC-2 in 1.0 mM Phosphate Buffer at 3 Temperatures

4. CONCLUSIONS

These studies showed that CC-2 has a strong affinity for sediments, has low water solubility, and is stable in the aqueous environment. An examination of Canal Creek sediments indicated that CC-2 was present at relatively high concentrations (77 + 5 ppm) but that TCA was not present. In laboratory studies, CC-2 concentrations remained stable in freshwater sediments over a 61-day exposure. There was no evidence for degradation of CC-2 to TCA in sediments or in overlaying water during this period. The solubility of CC-2 in mM phosphate buffer was investigated at three temperatures. Solubilities of 0.019 ± 0.002, 0.052 ± 0.003, and 0.084 ± 0.004 ppm (w/v) were obtained at 5, 15, and 25 °C, respectively.

All information collected indicate that CC-2 is relatively immobile, but persistent in the environment. There appears to be little potential for CC-2 to be taken up by aquatic organisms from the water column because of its affinity for sediments. However, other studies have shown that benthic organisms can bioaccumulate hydrophobic compounds from the sediments (Courtney and Langston 1978; McLeese et al. 1980). Benthic fauna may then serve as a contaminant source for fish through food chain transfer (Rubenstein et al. 1984). Thus, additional studies should be conducted to determine the toxicity of CC-2 and evaluate its potential to bioaccumulate in aquatic organisms.

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